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## Biology-Driven Library Design for Probe Discovery

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**Libraries of diverse small molecules are important to probe and drug discovery. The current trend toward building massive screening collections to support drug development, a special application of chemical biology, can limit their broader potential. Biology-driven construction methods (Wallace et al., 2011) are rapidly emerging to bring chemical libraries back on a viable path.**

When the label of a prescription medicine reads “inactive ingredients 98%,” it is telling you the vast majority of substances in the pills are inert, and that is an important and acceptable method of preparing a drug for administration. However, in compound libraries designed for chemical biology, inertness, with apt pharmacokinetics (PK)/pharmacodynamics or not, is an undesirable filler. Considerable effort is made to maximize the potential for bioactivity in modern screening libraries. Although no one claims to know exactly which compounds are bioactive in any particular system, data-driven models may help select the bright sparks from the dim bulbs.

The study conducted by Wallace et al. (2011 [this issue of *Chemistry & Biology*]) represents an important step toward establishing a dichotomy between therapeutic drug and biological probe discovery. For the most part, probe and drug development has historically been approached from the same canonical methodologies and central paradigms. However, with significantly different goals, it would seem logical to use divergent paths and starting points. For example, in library design, blanket application of Lipinski's rule of five (Ro5), and derivatives thereof, represents the status quo for compound

library selection. Ro5, intended to maximize the proportion of bioavailable small molecule agents, has been applied to guide most commercial and academic library development (Dolle, 2011). The reality today is that scientists seeking small molecules as probes to study a target of interest are relying on compound libraries designed to maximize favorable PK in human/animal subjects. Compounds based on peptide-like sequences (Kodadek, 2010), privileged structures, and natural products (Welsch et al., 2010) are examples of useful chemical probes that generally fall outside of the Ro5 criteria.

Clearly, the Ro5 along with many other general compound attributes are desirable for therapeutic drug discovery (Overington et al., 2006). Who would want their drug screening results to be dominated by unstable molecules with poor PK? Models developed to guide the population of screening libraries are needed, if not marginally to simplify high-throughput screening (HTS) logistics and costs, then importantly to accommodate novel technology and methodology. In this respect, the design of chemical libraries is more valuable than the sheer size. The results in Wallace et al. (2011) demonstrate, not unexpectedly, that the Ro5 may not always be the ideal filter for compounds

likely to be useful in chemical biology. A completely new set of compound selection principles may maximize the chemical space most relevant to non- or pretherapeutic applications. After all, the Wright brothers didn't include a pressurized cabin on their flying machine; why add drag before you even get off the ground? Wallace and colleagues move beyond generic property filters to develop models for bioactive molecule characteristics. This is an evolving concept in biology-driven library construction (Basu et al., 2011) that focuses on structural signatures instead of generic descriptors (such as calculated solubility partition coefficients, e.g., cLogP and molecular weight). In an examination of publicly available screening data sets, their Bayesian model showed promise in enriching for the most active hits.

Given the value of probe molecules to basic research, a fundamental rethinking of the methodologies used in such efforts is warranted. The Wallace et al. (2011) presents timely and immediately useful ideas for chemical biology. While the academic efforts to develop therapeutically relevant small molecules continue to show promise worldwide, their research to aid in the discovery of flexible and titratable tools (probes) that

complement the utility of genetic manipulations (siRNA, shRNA, zinc finger/TALEN, cDNA, etc.) holds substantial promise. As screening capability has become widespread and less dogmatic, academic HTS has embraced (to varying degrees) model organism-based assays in hosts such as bacteria, yeast, worm, and zebrafish. Model organisms have the advantage of accessible, well-characterized biology, expansive tool sets for assay design, and high degrees of conservation in many eukaryotic pathways allowing for relevant biology to be explored (Taylor et al., 2010). In this regard, the authors have utilized yeast as a basic platform to search for bioactive small molecules. Interestingly, they find that compounds with growth modifying phenotypes in yeast also exhibited activity in cultured cells from various eukaryotes.

Compact libraries of compiled bioactive small molecules such as LOAPC have proven to be of exceptional utility in chemical biology and are the household names of HTS and drug discovery. Presently, even as screening throughput can easily exceed 100,000 compounds per day in a modern HTS facility, the value of small, highly enriched compound libraries remains paramount. As these popular benchmark libraries have become a routine part of discovery campaigns, we should recognize that their existence was made possible by many years of research. Even though the bioactive library assembled in Wallace et al. (2011) contains much less annotation than collec-

tions such as LOPAC, the authors have demonstrated a method to accelerate mechanism-of-action (MOA) elucidation. Additionally, by leveraging the high throughput MOA mapping tools offered by model organisms such as yeast, the authors outlined a feasible path toward rapid annotation.

It is critical to realize that the haploinsufficiency profiling utilized in Wallace et al. (2011) to understand the basis of growth phenotype perturbation is just a fraction of what may be possible (Cong et al., 2011; Taylor et al., 2010). However, since cell viability is an inherently general phenotypic readout, it is important to emphasize that there may be potential pitfalls of such an approach. While profiling tools help guard against enrichment of unwanted artifacts of the chosen readout, new library construction models must still establish a proven track record as effective in delivering useful probes. We expect that future examples of compound enrichment schemes will include non-growth based readouts to strengthen the methodology.

Looking forward, we foresee new models of bioactivity enrichment playing an important role in probe discovery. Wallace et al. (2011) fits into a larger framework highlighted by Workman and Collins: defining the scientific paradigm of probe development (Workman and Collins, 2010). In light of the Workman and Collins "fit-for-purpose" definition of a useful biological probe, it is important to consider library design as being tailored to the individual needs of discovery proj-

ects. Wallace and colleagues have demonstrated how simple phenotypic screening of model organisms may be a path to rapidly build libraries targeting a custom-fit biology. Today, collections of approved drugs are the pinnacle of library enrichment for therapeutically endowed compounds (Huang et al., 2011). While these libraries continue to grow incrementally, probe-directed collections could achieve exponential expansion, potentially with important consequences for the progress of chemical biology.

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